Performance of Non-nasopharyngeal Sample Types for Molecular Detection of SARS-CoV-2

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KEYWORDS

• SARS-CoV-2 • COVID-19 • Molecular diagnostic • Nasopharyngeal swab • Non-NP respiratory specimen • Alternative specimen

KEY POINTS

- Although SARS-CoV-2 RNA can be detected in a variety of different body fluids, infectious virus particles are rarely recovered outside of the respiratory tract.
- The sensitivity of SARS-CoV-2 detection in non-nasopharyngeal samples varies with regards to the timeline of infection, disease severity, body site, and specimen collection method.
- Anterior nasal swabs, midturbinate swabs, combined nasal and oropharyngeal swabs, or saliva are acceptable alternatives to nasopharyngeal swabs for SARS-CoV-2 diagnosis and screening.
- The frequent shedding of viral RNA in stool provides rationale for using wastewater in public health surveillance. In contrast, SARS-CoV-2 is rarely detectable in blood and asymptomatic blood donors do not currently require screening.

INTRODUCTION

COVID-19 is predominantly a respiratory disease characterized by a dysregulated inflammatory response to a viral infection with presentations ranging from asymptomatic to multiorgan failure and death. The tropism of SARS-CoV-2, the virus that causes COVID-19, for various tissues is conferred by the widely expressed surface proteins, angiotensin-converting enzyme 2 (ACE2) and transmembrane serine protease 2 (TMPRSS2), which function as the receptor and coreceptor for cell entry,

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respectively. ACE2 is present on various cells of the respiratory tract, gastrointestinal (GI) tract, vasculature, and on epithelial cells of various other organs.[1] Owing to the potential for multiorgan system involvement and concerns for viral transmissibility, many studies have looked for detectable SARS-CoV-2 RNA in a variety of different body sites. Here, we review the findings and clinical utility of SARS-CoV-2 nucleic acid detection in commonly tested non-nasopharyngeal (NP) specimens.

RESPIRATORY TRACT SPECIMENS

Performance Characteristics of Non-nasopharyngeal Upper Respiratory Specimens

Nasopharyngeal swab (NPS) collection was initially assumed to be the preferred specimen type for SARS-CoV-2 detection. However, NPS collection is relatively invasive, requires trained health care workers wearing personal protective equipment (PPE), and is subject to sampling error. In addition, dedicated NPSs were in short supply during the pandemic. Consequently, alternative, non-NP upper respiratory tract (URT) sampling approaches including use of anterior nares (AN) swabs, midturbinate (MT) swabs, oropharyngeal (OP) swabs, and saliva were explored to mitigate one or more of the challenges associated with NPS collection (Table 1).

Saliva

Saliva was noted to be a promising specimen type early in the pandemic because SARS-CoV-2 was shown to be reproducibly detectable in the oral secretions of infected individuals, it is a noninvasive sample type that requires minimal supervision to obtain, and collection potentially minimizes health care personnel exposure to

Table 1
Recommendations of appropriate sample types for SARS-CoV-2 viral RNA testing

Sample Type	Diagnosis	Screening	Public Health Surveillance[a]	Not Clinically Useful
NP	×	×	×	
Saliva	×	×	×	
OP	×	×	×	
AN	×	×	×	
AN/MT	×	×	×	
Sputum	×			
ETS	×			
BAL	×			
Stool			× (Wastewater)	
Blood				×
CSF				×
Urine				×
Other blood fluids[b]				×

Abbreviations: BAL, bronchoalveolar lavage; CSF, cerebrospinal fluid; ETS, endotracheal secretions.

[a] Public health surveillance refers to testing of specimens that have no patient identification, are not reported to health care providers, and can therefore take place in non–CLIA-certified laboratories. The CDC and US Department of Health and Human Services oversees a collaborative effort for testing untreated wastewater and primary sludge in selected communities. Another example of public health surveillance includes genomic screening for novel viral variants.

[b] Includes reported studies of amniotic fluid, breast milk, conjunctival secretions, semen, and vaginal secretions.

infectious aerosols. In addition, saliva reduces the need for swabs and transport media. However, saliva is a complex matrix that can be difficult to work within the clinical laboratory. Automated sample-to-result platforms designed for swab collection tubes may not be amenable to use with saliva samples and saliva may require heat or chemical inactivation before testing.[2]

Overall test performance

Viral load is generally highest in saliva within the first week of infection.[3–5] Notably, SARS-CoV-2 RNA can be detected in saliva earlier and for a longer duration of time than NPS.[4] Multiple meta-analyses have compared SARS-CoV-2 RNA detection rates in saliva to NPS as well as assessed the impact of different saliva collection methods on test performance. Positivity rates across 4528 paired saliva and NPS samples were similar (88% [95% confidence interval (CI), 81%-93%] vs 94% [95% CI, 90%-98%] for saliva vs NPS, respectively).[2] Another meta-analysis showed similar nucleic acid amplification test (NAAT) pooled sensitivity for saliva versus NPS (sensitivity 83.2% [95% CI, 74.7%–91.4%] vs 84.8% [95% CI, 76.8%-92.4%]) and specificity (99.2% [95% CI, 95.2%–99.8%] vs 98.9% [95% CI, 97.4%-99.8%]), respectively.[6] Of note, saliva performed better than NPS in some studies highlighting the limitation of NPS as the gold standard.[5,7,8] Test characteristics varied substantially across different studies comparing saliva to NPS likely as a result of variability in the timing of testing relative to infection onset, severity of illness, and efficiency of nucleic acid extraction and amplification.

Collection method

Several different saliva collection methods have been assessed including passive drool/spit, coughed or deep-throat saliva, oral rinses, and fluid from oral cavity swabs. Saliva tests authorized by the Food and Drug Administration (FDA) for emergency use require collection tubes with stabilization or inactivation buffers. However, several studies have demonstrated high SARS-CoV-2 RNA stability in saliva collected in tubes without these additives, which may simplify specimen collection and reduce cost.[9,10]

Drool and spit methods. Recent meta-analyses reported that drool or spit protocols had an overall positive detection rate of 86% (95% CI, 78%–92%) compared with 95% for NPS (95% CI, 93%–97%) and was superior to oral fluid collected by swabs from the gumline.[2]

Saliva with coughing. Studies comparing coughed or deep-throat saliva had a positive detection rate of 94% compared with NPS, suggesting that these specimens may contain more virus than drool/spit saliva.[11,12] However, forced cough requires use of PPE to protect health care workers against potential infectious aerosols. Saliva that is excessively mucoid may lead to increased pipetting errors on automated systems necessitating a dilution or pretreatment/chemical digestion of the samples. Studies have shown comparable positivity and stability between undiluted or diluted saliva samples.[2]

Oral rinses. Few studies have evaluated oral rinses or gargles for SARS-CoV-2 detection. Saline gargles were suggested for hospitalized patients who were unable to produce sputum and for those patients who were unable to produce sufficient amounts of saliva.[13] Saline gargles appear to have comparable sensitivity to NPS collections for symptomatic patients.[14,15] In one study, the sensitivity and specificity of saline gargle were observed to be more than 90% and 98%, respectively, compared with NPS.[14] In contrast, another study reported a reduced sensitivity (63%) for oral rinses versus undiluted saliva (94.1%) relative to NPS. These studies differed in collection and testing methods. Oral rinses or gargles may be easier to collect than other forms of saliva, but additional data are required before this approach can be recommended.

Host factors

Host factors have also been shown to impact SARS CoV-2 test performance.

Symptomatic versus asymptomatic individuals. Symptomatic and asymptomatic individuals infected with SARS-CoV-2 are thought to harbor similar amounts of virus. However, paired comparisons of saliva to NPS for the detection of SARS-CoV-2 RNA in symptomatic and asymptomatic individuals have produced incongruent results potentially as a result of the timing of testing relative to symptom onset. One meta-analysis demonstrated high and comparable sensitivities of saliva in symptomatic and asymptomatic individuals (88% vs 87%, respectively), whereas NPS demonstrated higher sensitivity in symptomatic (96%) versus asymptomatic populations (73%).[2]

Pediatric patients. Relatively few saliva studies have been performed in the pediatric population and these reports differ widely in terms of sample size, collection, and testing methods.[2,16] The most robust studies, however, demonstrate comparable sensitivities between saliva and NPS in children (~82% vs 87%, respectively).[17,18] Overall, the subset of pediatric studies suggests that both NPS and saliva are acceptable sample types.

Nasal Swabs

Nasal swabs, when swabs are available, are also an attractive alternative to NPS because they are less invasive and can be collected by the patient. Nasal specimens are approved for use with many commercially available NAATs, but there are conflicting data on their test performance compared with NPS. Nasal swab specimens (including AN and MT) are generally obtained from both nares. The CDC's interim guidelines for COVID-19 clinical specimens[19] differentiate between AN and MT collection by the distance of swab insertion into the nostril, but these terms are often used interchangeably in studies comparing their performance to NPS.

A recent meta-analysis showed that nasal swabs (either AN or MT) had a lower positive detection rate of 82% (95% CI, 73%–90%) compared with 98% (95% CI, 96%–100%) for NPS, and there was modest agreement (79%) between the 2 specimen types. Detection with nasal swabs was highest in individuals with symptoms $\leq$ 7 days (88% [95% CI, 74%–95%]).[2]

As observed with saliva collection, substantial heterogeneity was observed in studies comparing nasal and NPS test performance. In addition to the timing of testing, swab type has also been shown to affect test sensitivity. Specifically, foam or flocked nasal swabs performed better than unflocked or polyester swab specimens (percent positivity 90%, [95% CI, 81%–97%] versus 77% [95% CI, 55%–93%], respectively),[20,21] whereas the person collecting the sample (self vs health care worker) and use of transport media (dry swab vs diluted) do not appear to impact detection rates.[20,21,2]

Oropharyngeal and Dual Anterior Nares/Oropharyngeal Swabs

A meta-analysis of OP swab and NPS samples in symptomatic patients found similar positivity rates (84% [95% CI, 57%–100%] vs 88% [95% CI, 73%–98%], respectively), but the overall agreement between the 2 sample types was only 68% (95% CI, 36%–93%).[2] Two additional meta-analyses showed that combining both OP and AN swabs in a single collection tube improves the rate of SARS-CoV-2 detection by molecular methods (sensitivity 97% and specificity 99%).[2,22] In addition, supervised self-collected OP/AN swabs had comparable sensitivity to Health care worker (HCW)-collected OP/AN swabs using 3 different molecular testing platforms.[23]

Performance Characteristics of Lower Respiratory Tract Specimens

The lower respiratory tract (LRT) is considered to be the most sensitive anatomic site for SARS-CoV-2 RNA detection,[24] possibly due to reports suggesting that higher levels of RNA are present in this anatomic compartment.[25,26] However, this observation may be biased by severe presentations, which presumably are associated with higher virion burden, were more likely to have LRT testing performed. Viral RNA loads and infectious particles peak in the first week of infection in the LRT (**Fig. 1**),[27–29] but the shedding of viral RNA and infectious virus particles can be prolonged in this compartment.[25,28] Prolonged shedding is thought to be more pronounced in severe disease and in the immunocompromised. These patients may yield positive test results for several months beyond the resolution of symptoms,[25,26,30] and the clinical and infection control significance of this is not fully known. Additional limitations of LRT testing include the need for clinical laboratories to validate additional sample

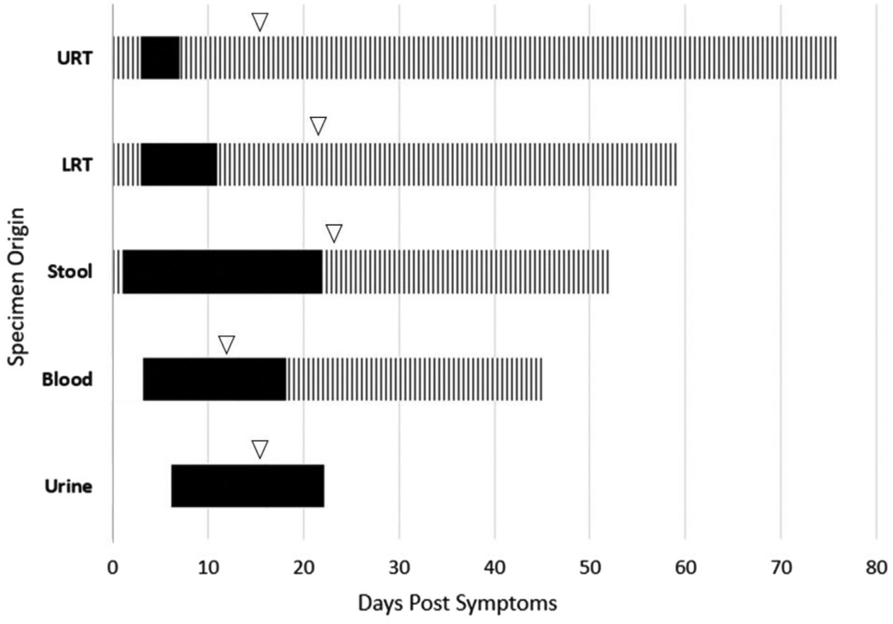

Fig. 1. Illustration of the estimated reported ranges of detection of SARS-CoV-2 RNA in specimens from different anatomic regions in symptomatic patients. Ranges of detection are approximations according to several meta-analyses of SARS-CoV-2 detection by RT-PCR.[25–27,29] This datum has not been standardized between studies. The peak Ct range (*black area*), average duration of detection (*arrowhead*), and total reported detectable range (*black + vertical line areas*) of SARS-CoV-2 RNA are averages of published data from the same references and were inferred in these cases from studies with serial RT-PCR testing and reported Ct values for these specimen types. Upper and lower respiratory tract specimens peak most often in the first week, whereas the peak range for non-respiratory specimens is not well-defined. Note that detection of viral RNA in blood and urine is infrequent, and average and extreme ranges overlap. Specific specimen types were omitted due to a relative lack of quality, high power data for meaningful comparisons.

types that may not be covered under emergency use authorizations for commercially available SARS-CoV-2 NAATs. Common LRT sampling strategies are also associated with risk for aerosol generation and some (eg, bronchoscopy) are invasive.

Few studies have directly compared the diagnostic yield of various LRT specimen types to one another or to NPS collected from the same patient at the time. In one study of greater than 1000 patients where exposure history, symptoms, and radiology were used as a reference method to confirm COVID-19, the highest viral RNA detection rates were in bronchoalveolar lavage (BAL) specimens (93%), followed by sputum (72%) and then nasal swabs (63%).[31]

Sputum

Sputum can be collected during voluntary expectoration or by induction. However, COVID-19 patients may not have a productive cough, and sputum induction has generally been avoided because of the risk of generating infectious aerosols. Thick mucus might also add to the difficulty in isolating nucleic acids in some specimens. Classic nucleic acid isolation techniques in sputum have included treatments with reducing agents and mucolytic enzymes to reduce viscosity and increase nucleic acid yield. Manufacturer-approved sterile collection tubes should also be used for molecular testing, as certain synthetic polymers can bind nucleic acid and interfere with the yield of extraction.

Endotracheal Aspiration

Endotracheal aspiration (ETA) specimens can be acquired from intubated patients or through a tracheostomy tube. To collect this specimen type, a catheter is placed through the endotracheal tube, and secretions are usually aspirated into a sterile preattached specimen trap. ETAs are sometimes collected when more invasive testing strategies are not possible and may be less likely to produce aerosols compared with bronchoscopy. ETA samples are thought to have similar sensitivities compared to sputum, but relatively little performance data for ETA exist.[31,32]

Bronchoalveolar Lavage and Bronchial Washings

BAL sampling is performed during minimally flexible bronchoscopy by instilling a specific amount of saline and aspirating this volume for testing.[33] For example, a lavage volume of 100 mL samples approximately 1.5% to 3.0% of the lung, or approximately one million alveoli. BAL may be performed with or without established intubation and many centers tried to avoid these procedures because of aerosol generation. However, reports of increased SARS-CoV-2 detection sensitivity of this specimen type provide diagnostic rationale, especially in the case of pneumonia with negative URT testing and high radiologic and/or clinical suspicion of COVID-19.[34] BAL sampling has been shown to have the highest rate of detection compared with all other samples in systematic comparison studies as well as in late clinical detection and persistent disease.[26,31] BAL testing may also be particularly useful when coinfections need to be ruled out with high confidence.

Summary of Current COVID-19 Diagnostic Guidelines for Respiratory Specimens

Owing to convenience, URT testing is preferred as the first-line test. However, LRT testing may also be useful, especially when URT testing is negative but a high clinical suspicion for COVID-19 pneumonia remains. Current IDSA guidelines recommend collection of NPS, MTS, ANS, saliva, or a combined AN/OP swabs rather than OP swab alone as a first test for all symptomatic persons.[35]

NONRESPIRATORY SPECIMENS

Stool

Following the respiratory tract, the GI system is the next most affected organ system by COVID-19, which is believed to be related to the viral tropism of various cell types in the GI tract.[1] Several studies estimate that GI symptoms in COVID-19 patients may be present in approximately 10% to 50% of patients.[36,37] The presentation of GI symptoms ranges from asymptomatic to severe disturbances, which in some cases is reported independent of the severity of the respiratory disease.[28] SARS-CoV-2 RNA has been detected from various GI specimens including gastric lavage stool, and anorectal swabs, but stool is the most reported specimen type. Certainly, collection and reverse transcription–polymerase chain reaction (PCR) needs to be carefully standardized to ensure meaningful data in this highly variable sample type. There are many natural constituents of stool that degrade viral RNA and inhibit PCR reactions, including degradative enzymes, bile salts, and dietary polysaccharides.

Despite the natural challenges with this specimen type, viral RNA has been detected in stool over a broad timeframe regardless of GI symptoms or disease severity.[25,26] The detection of viral RNA in stool does not seem to correlate with respiratory viral RNA detection or vice versa.[25,28,38] There is significant heterogeneity between studies and meta-analyses with regards to clinical sensitivity (approximate range, 30%–60%) and detection timeframes (see **Fig. 1**).[25,26,37,38] In one meta-analysis, there was reported viral shedding in several studies at least 4 weeks after symptom onset.[25] Patients without GI symptoms may still have detectable viral RNA in a stool sample much beyond the resolution of COVID-19 symptoms.[26,29,36] A minority of specimens with detectable viral RNA in stool also has yielded infectious virus in culture, suggesting that fecal-oral transmission of COVID-19 may be theoretically possible even though most of these specimens are considered noninfectious.[31,38] Owing to the relatively high abundance of viral RNA shedding in stool samples, several population studies have also shown that wastewater and sewage may serve as a useful sample for population screening, which has been used in several public health surveillance strategies.[39] This approach to a massively "pooled" specimen may be useful for predicting outbreaks on the population level.

Blood

As with stool, meta-analyses of the detection of SARS-CoV-2 RNA in serum or plasma have significant heterogeneity and this specimen type is not recommended for diagnostic testing.[19,40]When compared with all respiratory sample types, the clinical sensitivity of blood specimens for COVID-19 infection is consistently much lower (~0%-45%), and positive detection appears to correlate with disease severity and mortality in a few reports.[26,29,41] When testing blood, molecular tests should exclude samples with residual, well-described, and ubiquitous PCR inhibitors from their protocols, such as heme and heparin.

Viral RNA detection surprisingly has been detected up to 60 days in serum,[25] but most studies report an average detection timeframe of 3 to 18 days (see **Fig. 1**).[26] Despite the potential prolonged detection of viral RNA in these specimen types, no live virus has been successfully isolated from serum in these reports.[26,28] Recently, a massive study of 258,000 blood donations showed very infrequent detection of SARS-CoV-2 RNA (1.16/100,000), and no infectivity was demonstrated in cell culture.[42] Considering an extremely low risk of COVID-19 transmission from blood, FDA does not currently require screening routine asymptomatic blood donors for SARS-CoV-2 or after 14 days of resolution of COVID-19 symptoms.[43]

Other Body Fluids

SARS-CoV-2 testing has been performed on the body fluids that are discussed in **Table 1**, but data currently exist as case reports without paired comparisons to a reference method or standardized molecular detection methods. None of the fluids discussed in **Table 1** are currently considered clinically useful for the detection of SARS-CoV-2.

Cerebrospinal fluid (CSF) testing has been reported in several studies and analyzed in meta-analyses, with rare reports detected viral RNA.[26] It is not clear if these instances represent a true positive, viremia with CSF blood contamination, or breach of the blood-brain barrier due to systemic inflammation, or other vascular pathology. There appears to be no clinical indication for CSF sampling in COVID-19 patients, yet some laboratories may still offer this testing as a validated specimen type. There is a similar scenario with urine, where little to no detection has been reported.[44,45] Detected viral RNA could represent primary renal involvement or viremia with widespread vascular pathology. Of note, ACE2 and TMPRSS2 are coexpressed in renal tubules and podocytes,[46] and primary COVID-19–associated kidney pathology has been proposed.[47] There is also inconclusive or conflicting evidence for the true involvement of SARS-CoV-2 in amniotic fluid, breast milk, conjunctival secretions, semen, and vaginal secretions.[26]

SUMMARY

SARS-CoV-2 viral RNA shedding is temporally dynamic and differs between respiratory and distant anatomic sites. Standardized collection processes and appropriate testing rationale are essential, regardless of specimen type. The vast majority of test performance data come from assessments of various URT specimen types. URT samples are easy to obtain and accurate in comparison with NP testing, but it should be noted that NP testing is an imperfect gold standard and there may be circumstances where alternatives are preferred. There is also clear utility in testing the LRT, especially when URT testing is negative and there is high clinical suspicion for COVID-19 pneumonia. Stool testing is not as clinically sensitive in COVID-19 patients as respiratory specimens, but studies of sewage show continual promise as a population-level screening tool. The presence of SARS-CoV-2 viral RNA in blood and other body fluids appears to be transient in the most severely ill patients, shows great variability, and should not be performed to track disease progression. A shared knowledge of the limitations of molecular tests is also clinically essential as we face a landscape of novel variants with unpredictable testing parameters. Constant adaptation to these viral dynamics will be required in addition to higher quality data for emerging detection technologies.

CLINICS CARE POINTS

- Non-nasopharyngeal upper respiratory tract specimens such as nasal swab or saliva may be used for molecular detection of SARS-CoV-2.
- Various factors including timing of onset of symptoms, host factors, and method of collection may affect test sensitivity for non-nasopharyngeal specimens.
- Lower respiratory tract specimens like BAL are a useful diagnostic specimen in patients with COVID-19 pneumonia.-Testing of stool and other non-respiratory specimens has a limited role in the diagnosis of COVID-19.

- Testing of stool and other non-respiratory specimens has a limited role in the diagnosis of COVID-19.
- Further studies are needed to understand the effect of SARS-CoV-2 variants on the detection rate of non-nasopharyngeal specimens.

DISCLOSURE

The authors have nothing to disclose.

REFERENCES

1. Salamanna F, Maglio M, Landini MP, et al. Body Localization of ACE-2: on the Trail of the Keyhole of SARS-CoV-2. Front Med 2020;7:594495.
2. Lee RA, Herigon JC, Benedetti A, et al. Performance of saliva, oropharyngeal swabs, and nasal swabs for SARS-CoV-2 molecular detection: a systematic review and meta-analysis. J Clin Microbiol 2021;59(5).
3. Teo AKJ, Choudhury Y, Tan IB, et al. Saliva is more sensitive than nasopharyngeal or nasal swabs for diagnosis of asymptomatic and mild COVID-19 infection. Sci Rep 2021;11(1):3134.
4. Williams E, Bond K, Zhang B, et al. Saliva as a Noninvasive specimen for detection of SARS-CoV-2. J Clin Microbiol 2020;58(8).
5. Wyllie AL, Fournier J, Casanovas-Massana A, et al. Saliva or nasopharyngeal swab specimens for detection of SARS-CoV-2. N Engl J Med 2020;383(13):1283–6.
6. Butler-Laporte G, Lawandi A, Schiller I, et al. Comparison of saliva and nasopharyngeal swab nucleic acid amplification testing for detection of SARS-CoV-2: a systematic review and meta-analysis. JAMA Intern Med 2021;181(3):353–60.
7. Hanson KE, Barker AP, Hillyard DR, et al. Self-collected anterior nasal and saliva specimens versus healthcare worker-collected nasopharyngeal swabs for the molecular detection of SARS-CoV-2. J Clin Microbiol 2020;58(11).
8. Kojima N, Turner F, Slepnev V, et al. Self-collected oral fluid and nasal swab specimens demonstrate comparable sensitivity to Clinician-collected nasopharyngeal swab specimens for the detection of SARS-CoV-2. Clin Infect Dis 2021;73(9).
9. Griesemer SB, Van Slyke G, Ehrbar D, et al. Evaluation of specimen types and saliva stabilization Solutions for SARS-CoV-2 testing. J Clin Microbiol 2021;59(5).
10. Ott IM, Strine MS, Watkins AE, et al. Stability of SARS-CoV-2 RNA in Nonsupplemented saliva. Emerg Infect Dis 2021;27(4):1146–50.
11. Procop GW, Shrestha NK, Vogel S, et al. A Direct comparison of Enhanced saliva to nasopharyngeal swab for the detection of SARS-CoV-2 in symptomatic patients. J Clin Microbiol 2020;58(11).
12. Rao M, Rashid FA, Sabri F, et al. Comparing nasopharyngeal swab and early Morning saliva for the identification of severe acute respiratory Syndrome coronavirus 2 (SARS-CoV-2). Clin Infect Dis 2021;72(9):e352–6.
13. Saito M, Adachi E, Yamayoshi S, et al. Gargle lavage as a Safe and sensitive alternative to swab samples to Diagnose COVID-19: a case report in Japan. Clin Infect Dis 2020;71(15):893–4.
14. Goldfarb DM, Tilley P, Al-Rawahi GN, et al. Self-collected saline gargle samples as an alternative to health care worker-collected nasopharyngeal swabs for COVID-19 diagnosis in outpatients. J Clin Microbiol 2021;59(4).

15. Kandel CE, Young M, Serbanescu MA, et al. Detection of severe acute respiratory coronavirus virus 2 (SARS-CoV-2) in outpatients: a multicenter comparison of self-collected saline gargle, oral swab, and combined oral-anterior nasal swab to a provider collected nasopharyngeal swab. Infect Control Hosp Epidemiol 2021;1–5.
16. Chong CY, Kam KQ, Li J, et al. Saliva is not a useful diagnostic specimen in children with Coronavirus Disease 2019. Clin Infect Dis 2021;73(9).
17. Al Suwaidi H, Senok A, Varghese R, et al. Saliva for molecular detection of SARS-CoV-2 in school-age children. Clin Microbiol Infect 2021;27(9):1330–5.
18. Yee R, Truong TT, Pannaraj PS, et al. Saliva is a promising alternative specimen for the detection of SARS-CoV-2 in children and Adults. J Clin Microbiol 2021;59(2).
19. Interim CDC. Guidelines for collecting, Handling, and testing clinical specimens for COVID-19. 2020. Available at: https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html. Accessed April 22nd, 2021.
20. Tu YP, Jennings R, Hart B, et al. Swabs collected by patients or health care workers for SARS-CoV-2 testing. N Engl J Med 2020;383(5):494–6.
21. Hart B, Tu YP, Jennings R, et al. A comparison of health care worker-collected foam and polyester nasal swabs in convalescent COVID-19 patients. PLoS One 2020;15(10):e0241100.
22. Tsang NNY, So HC, Ng KY, et al. Diagnostic performance of different sampling approaches for SARS-CoV-2 RT-PCR testing: a systematic review and meta-analysis. Lancet Infect Dis 2021;21(9):1233–45.
23. LeBlanc JJ, Heinstein C, MacDonald J, et al. A combined oropharyngeal/nares swab is a suitable alternative to nasopharyngeal swabs for the detection of SARS-CoV-2. J Clin Virol 2020;128:104442.
24. Price TK, Bowland BC, Chandrasekaran S, et al. Performance characteristics of severe acute respiratory Syndrome coronavirus 2 RT-PCR tests in a single health system: analysis of >10,000 results from three different Assays. J Mol Diagn 2021;23(2):159–63.
25. Cevik M, Tate M, Lloyd O, et al. SARS-CoV-2, SARS-CoV, and MERS-CoV viral load dynamics, duration of viral shedding, and infectiousness: a systematic review and meta-analysis. Lancet Microbe 2021;2(1):e13–22.
26. Stanoeva KR, van der Eijk AA, Meijer A, et al. Towards a sensitive and accurate interpretation of molecular testing for SARS-CoV-2: a rapid review of 264 studies. Euro Surveill 2021;26(10).
27. Walsh KA, Jordan K, Clyne B, et al. SARS-CoV-2 detection, viral load and infectivity over the course of an infection. J Infect 2020;81(3):357–71.
28. Wolfel R, Corman VM, Guggemos W, et al. Virological assessment of hospitalized patients with COVID-2019. Nature 2020;581(7809):465–9.
29. Zhurakivska K, Troiano G, Pannone G, et al. An Overview of the temporal shedding of SARS-CoV-2 RNA in clinical specimens. Front Public Health 2020;8:487.
30. Zapor M. Persistent detection and infectious potential of SARS-CoV-2 virus in clinical specimens from COVID-19 patients. Viruses 2020;12(12).
31. Wang W, Xu Y, Gao R, et al. Detection of SARS-CoV-2 in different types of clinical specimens. Jama 2020;323(18):1843–4.
32. Huang Y, Chen S, Yang Z, et al. SARS-CoV-2 viral load in clinical samples from Critically ill patients. Am J Respir Crit Care Med 2020;201(11):1435–8.
33. Interim Laboratory CDC. Biosafety guidelines for Handling and processing specimens associated with Coronavirus disease 2019 (COVID-19). 2021. Available at:

https://www.cdc.gov/coronavirus/2019-ncov/lab/lab-biosafety-guidelines.html. Accessed May 2021.

34. Winichakoon P, Chaiwarith R, Liwsrisakun C, et al. Negative nasopharyngeal and oropharyngeal swabs do not Rule out COVID-19. J Clin Microbiol 2020;58(5).
35. Hanson KECA, Arias CA, Hayden MK, et al. Infectious diseases Society of America guidelines on the diagnosis of COVID-19: molecular diagnostic testing. Infectious Diseases Society of America. 2020. *Version 200.* 2020;Available at. https://www.idsociety.org/practice-guideline/covid-19-guideline-diagnostics/. Accessed May 22 2021.
36. Parasa S, Desai M, Thoguluva Chandrasekar V, et al. Prevalence of gastrointestinal symptoms and fecal viral shedding in patients with coronavirus disease 2019: a systematic review and meta-analysis. JAMA Netw Open 2020;3(6):e2011335.
37. Rokkas T. Gastrointestinal involvement in COVID-19: a systematic review and meta-analysis. Ann Gastroenterol 2020;33(4):355–65.
38. van Doorn AS, Meijer B, Frampton CMA, et al. Systematic review with meta-analysis: SARS-CoV-2 stool testing and the potential for faecal-oral transmission. Aliment Pharmacol Ther 2020;52(8):1276–88.
39. National CDC. Wastewater surveillance system (NWSS). 2021. Available at: https://www.cdc.gov/coronavirus/2019-ncov/cases-updates/wastewater-surveillance.html. Accessed May 2021.
40. FDA. Coronavirus Disease 2019 testing Basics. Accessed December 17, 2021. 2021.
41. Fajnzylber J, Regan J, Coxen K, et al. SARS-CoV-2 viral load is associated with increased disease severity and mortality. Nat Commun 2020;11(1):5493.
42. Bakkour S, Saa P, Groves JA, et al. Minipool testing for SARS-CoV-2 RNA in United States blood donors. Transfusion 2021;61(8):2384–91.
43. FDA. Updated Information for Blood Establishments Regarding the COVID-19 Pandemic and Blood Donation. 2021. Available at: https://www.fda.gov/vaccines-blood-biologics/safety-availability-biologics/updated-information-blood-establishments-regarding-covid-19-pandemic-and-blood-donation. Accessed May 23, 2021.
44. Bwire GM, Majigo MV, Njiro BJ, et al. Detection profile of SARS-CoV-2 using RT-PCR in different types of clinical specimens: a systematic review and meta-analysis. J Med Virol 2021;93(2):719–25.
45. Johnson H, Garg M, Shantikumar S, et al. COVID-19 (SARS-CoV-2) in Non-Airborne body fluids: a systematic review & Meta-analysis. Turkish J Urol 2021;47(2):87–97.
46. Zou X, Chen K, Zou J, et al. Single-cell RNA-seq data analysis on the receptor ACE2 expression reveals the potential risk of different human organs vulnerable to 2019-nCoV infection. Front Med 2020;14(2):185–92.
47. Nasr SH, Kopp JB. COVID-19-Associated collapsing glomerulopathy: an emerging entity. Kidney Int Rep 2020;5(6):759–61.